

# EFFICACY OF THE NOVEL OPTIPREP—UWS GRADIENT FOR HUMAN ISLET PURIFICATION

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# Consistent human islet isolation and purification success....

- ... is hampered by the variability of donor and procurement factors
- However, by simple changes in (pre-) purification solutions
  - substantial progress in purification has been made in the past
  - suggesting that considerable scope still exists for further improvement of the purification outcome
- We recently developed a ~100% efficient density gradient of OptiPrep\* in University of Wisconsin solution (UWS) in the difficult pig model
- This success prompted us to test this gradient during 5 consecutive human islet isolations

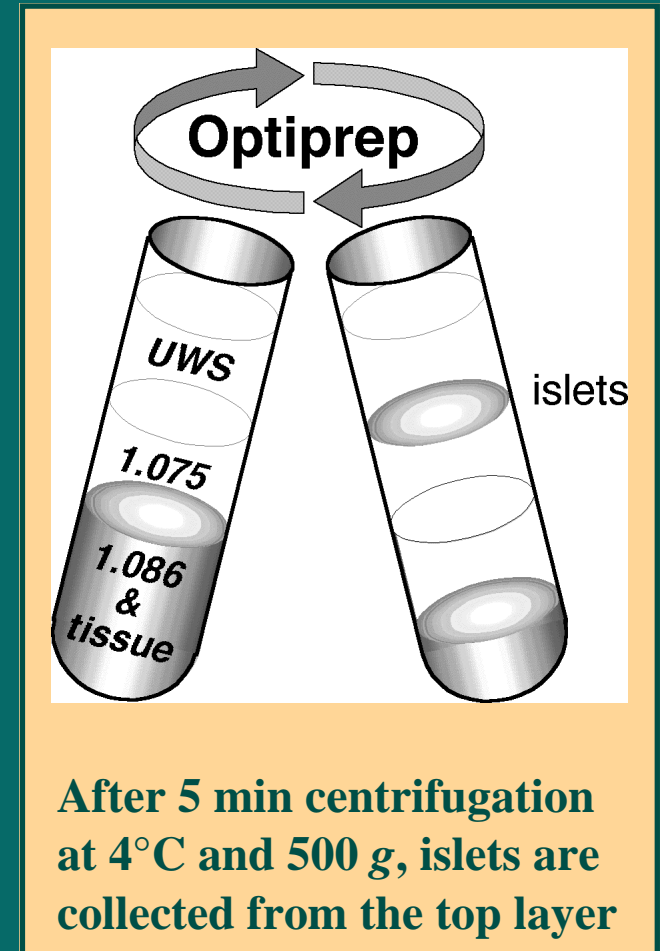
\* OptiPrep is a 60% iodixanol solution produced by Nycomed (Oslo, Norway)

# PROCEDURE

- **Pancreases**
  - **distantly procured from multiorgan (27–57 y) donors, and**
  - **cold preserved with UWS for ~12 h, were**
  - **digested with Liberase-HI in Hanks by the automated method**
- **The digest was collected with cold RPMI, and a sample of the digest was taken for these pilot experiments**
  - » **the remainder was used in non-related experiments**
- **Next, the tissue was incubated 60 min in UWS on ice**
  - » **an aliquot was taken for assessment**
- **Half of the prep was loaded in an 1.086-1.075-UWS gradient**
  - **the other half was saved for optional testing of other densities**

# RECIPE FOR THE OPTIPREP—UWS GRADIENT

- OptiPrep is a 60% iodixanol in H<sub>2</sub>O solution
- Working OptiPrep solution (WOP) is prepared by mixing equal volumes of
  - OptiPrep, and
  - double-strength UWS (UWS 2x)
- The 1.086 bottom is prepared by mixing
  - 30 ml digest (in UWS) and
  - 10 ml WOP
- The 1.075 (or 1.070) barrier layer is prepared by mixing
  - 5 ml WOP and
  - 22.6 ml (or 28.3) UWS



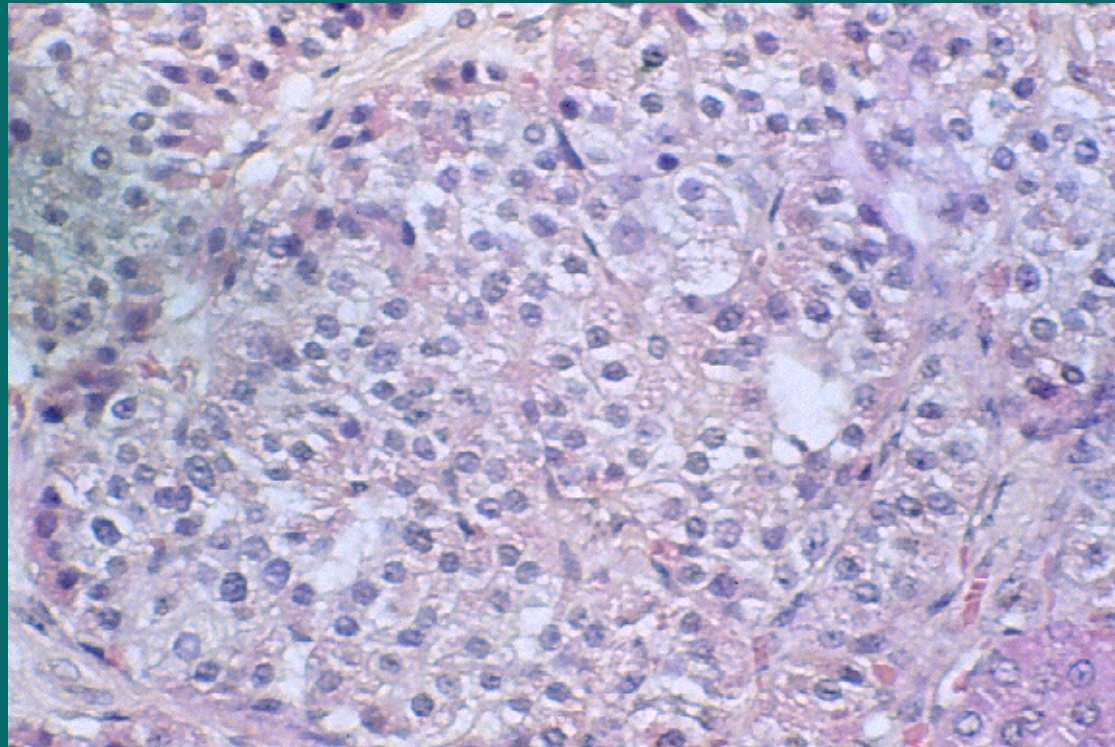
# RESULTS...

- During all the first 4 experiments the 1.086-1.075-UWS gradient was successful (> 80% purity and recovery)
- In the last experiment, purity was 35% at the top, but again > 80% purity and recovery were obtained by using an 1.070 barrier (not a 1.075) with the other half of the digest prep
- On average:

	DIGEST	TOP GRADIENT
IEQs	15287 ± 3712	12766 ± 3129
Recovery %		83 ± 2
Diameter μm	238 ± 22	204 ± 13
Purity %		89 ± 3

## RESULTS — cont'd

- **Viability of the islets was corroborated histologically 1 to 3 wk after transplantation under the kidney capsule in 3 nude mice**



# Powerful Tool for Human Islet Purification (?)

- Thus, the consistent high efficacy of the simple OptiPrep-UWS gradient in this pilot & the favourable characteristics such as
  - the mild hyperosmotic conditions (~360 mOsm)
  - a low endotoxin content
  - the clinical safety-tested components
- » suggest that the gradient (with densities like 1.086–1.075–1.070–UWS) may become a new powerful tool for human islet purification
- Nycomed plans to produce the working Optiprep-UWS this year